Use of Exo-polygalacturonase to Improve Extraction Yields of Alginic Acid from Sea Mustard (Undaria pinnatifida)

- Research Note -

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Abstract

Exo-polygalacturonase (EPG) from Rhizopus sp. was applied to the extraction of alginic acid from sea mustard to increase extraction yield. EPG digestion was examined under distinct conditions within temperatures from 25°C to 50°C, pH 5 to 9, and treatment times from 0 to 36 hr. The optimal conditions for alginic acid extraction with EPG were: pH 7.0 at 30°C for 24 hrs. The EPG hot water extraction yield was 3.4 times higher yield than hot water extraction alone. Using EPG to extract alginic acid from sea mustard should be considered a viable alternative to conventional extraction, with the advantage of reducing hazardous wastes such as strong acid and alkali solutions.

Key words: alginic acid extraction, exo-polygalacturonase, sea mustard

INTRODUCTION

Alginic acid is a linear polysaccharide that is commercially extracted from many strains of marine brown seaweed. Alginic acid is a co-polymer of D-mannuronic acid and L-guluronic acid, in which the uronic acids are arranged in a block fashion in the polymeric chain (1). Alginites have widespread applications in the pharmaceutical and food industries, owing to their ability to form viscous solutions at relatively low concentrations and to form gels with Ca²⁺. Alginic acid from sea mustard can be extracted with hot water in a sodium carbonate solution, or by alternation of acidic and alkaline treatments (2). The highest industrial extraction yields are obtained utilizing sodium carbonate solutions or acidic and alkaline treatments. However, the strong caustic and acidic chemicals produce hazardous toxic wastes that threaten the environment and damage reactors used in the extraction process. Hot water extraction is safer and produces no toxic chemicals, but is inefficient because of low yields (2). In this study, we evaluated the use of enzymatic digestion of sea mustard, using exo-polygalacturonase (EPG), for increasing the yield of alginic acid from hot water extraction.

EPG, a protease, is normally produced by microorganisms to facilitate plant degradation (3-7). EPG cleaves side chains of neutral sugars of pectins that are linked to cellulose or are residues of homogalacturonan. Applications using EPG include pectin production (8), isolation of single cells from vegetable food material (7,9), and the isolation of protoplasts from plant cells (10). Sea mustard alginic acid is a structural component of the cell wall that is esterified to cellulose or hemicellulose. EPG randomly hydrolyses terminal α-1,4-glucoside bonds of D-guluronic acid, thereby isolating D-guluronic acid. Therefore, the use of EPG may be expected to increase the yields from hot water extraction of alginic acid from sea mustard.

MATERIALS AND METHODS

Materials

Dried sea mustard (Undaria pinnatifida) was purchased from a local food market in Seoul, Korea; ground with a hammer mill, and screened through an 80-mesh sieve. EPG (Macerozyme R-10) from Rhizopus sp. was obtained from Yakult Co. (Tokyo, Japan). All other chemicals were analytical grade.

Extraction of alginic acid by hot-water solubilization method

The extraction of alginic acid from sea mustard was performed as described by Nishide et al. (2), except that the formaldehyde treatment was replaced by EPG digestion for the enzymatic extraction. For non-enzymatic extraction, 5 g of the ground sea mustard was placed in a stoppered flask containing 50 mL of a 3.7% formaldehyde solution and maintained at 30°C overnight.

For the EPG treatment, 5 g of sea mustard was incubated in 50 mL of various pH buffer solutions containing 50 mg of EPG (3 U/mg of protein). To determine the pH effect
on the EPG alginic acid extraction, the following buffers were used: 20 mM acetate buffer (pH 5.0), 20 mM sodium phosphate buffer (pH 6.0), 20 mM potassium phosphate buffer (pH 7.0), 20 mM Tris-HCl buffer (pH 8.0), and 20 mM glycine buffer (pH 9.0). The effect of alginic acid extraction with EPG was determined at different temperatures (25, 30, 35, 40, 45 and 50°C) and at different durations of time (0, 4, 8, 12, 16, 20, 24, 28, 32 and 36 hr).

Following each enzymatic or non-enzymatic extraction under a given condition, each reaction mixture was diluted with 100 mL of distilled water and hot water extracted by stirring at 100°C for 4 hrs. After filtering through a hemp cloth, the filtrates were dialyzed by cellulose membrane (Avg. flat width 43 mm; Avg. diameter 27 mm; Capacity approx. 175 mL/lit; cutoff size > M.W. 12,000: Sigma Chemical Co.) in distilled water, and filtered through a filter paper (Toyko No.2). The dialyzed inner fluid was concentrated to one-fourth of the initial volume using a rotary evaporator (NE-1S, Tokyo Rikakikai Co., Ltd.). Ethanol was then added to make an 80% final concentration. The precipitated alginate gel was obtained by centrifugation at 3,000 × g for 10 min. The pellet was rinsed with ethanol and then with acetone, and centrifuged at 3,000 × g for 5 min. The alginate pellet was dried to powder at 50°C for 12 hrs and weighed. The yield was calculated as the percentage of alginic acid extracted from each 5 g sample. Recovery rate was calculated as the weight ratio of pure alginate extracted to that of the pellet weight. Each experiment was performed in duplicate and the value reported was an average of the two data.

**Determination of purity of alginic acid**

Purity of extracted alginic acid was determined by the m-hydroxydiphenyl method (11), and expressed as % of uronic acid in the total sample.

**RESULTS AND DISCUSSION**

The effect of temperature on EPG activity was determined from 24 h alginic acid extraction yields at 25, 30, 35, 40, 45 or 50°C at pH 7 (Fig. 1). The highest yield of alginic acid by EPG treatment was obtained at 30°C, which is presumed to be the optimal temperature for EPG extraction.

The greatest alginic acid yield from sea mustard was achieved at pH 7.0 after 24 h incubation with EPG (Fig. 2). EPG is stable at pH 5 and 6 (12), but the optimal pHs for pectin extraction with EPG from apple and pear pomace are 7.0 and 7.8, respectively (13,14). The pH of incubation solutions alters the electrical charges of both enzyme and substrate, and profoundly affects the recognition of the active site of enzymes and the separation of products after the reaction. Although we determined that pH 7.0 was optimal under the conditions we used, we cannot exclude the possibility that different buffers would behave differently and that a different pH would produce maximum yields if a different buffer was used. Therefore, further experiments using a variety of buffers over a broad pH range is needed to determine the optimal pH for EPG extraction.

The maximum yield of alginic acid was obtained after 24 h incubation with EPG (Fig. 3). EPG is a macerating enzyme due to its hydrolytic activity on propectin in plant cell walls. Pectin extraction from apple and pear pomace by EPG was maximized with incubation times of 60 and 36 hours, respectively (13,14). Although the mechanism is unknown, the hydrolytic activity of EPG on glucosidic bonds in sea mustard can be inferred from its effectiveness in alginic acid extraction.

As shown above, the highest extraction yield (8.1% of alginic acid from sea mustard) was obtained with EPG treatment for 24 hrs at pH 7 and 30°C. In contrast, there was only a 2.4% yield from the non-enzymatic hot water extraction (Table 1). The increased yield with the EPG treat-
application of EPG in a hot water extraction system should be considered to be an appropriate alternative method for the extraction of alginate from sea mustard.

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REFERENCES


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